## Recommendations

1. Animal models. In veiw of the difficulties with field trials, there continues to be the need for the development of animal systems which can be closely correlated with serological responsiveness on the one hand and protective efficacy in man on the other. Such an animal model or test system is not currently available.

2. The available data regarding immune responses should be reflected in recommendations for use of the product.

3. Plague vaccine U.S.P. (E medium) is judged by the Panel to be safe and effective. Revised labeling for civilian use of plague vaccine, following an amendment of license in November 1974 has not been seen by the Panel and remains to be reviewed.

## Basis for Recommendations

Judgment of efficacy in the case of plague vaccine is based upon epidemiological evidence obtained in military populations rather than formal field trials or serological data directly correlated with protection in man. Nonetheless, the Panel believes that the plague vaccine as prepared for military use should be classified in Category I because the available data provide evidence of efficacy. The Panel believes that the data obtained from this epidemiologic investigation is adequate to substantiate effectiveness in this case.

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SPECIFIC PRODUCT REVIEW

PLAGUE VACCINE MANUFACTURED BY CUTTER LABORATORIES, INC.

1. Description. This is a suspension of whole plague bacilli (Yersinia pestis, strain 195/P), formalin killed in a concentration of 2 thousand million organisms per ml. The suspending medium contains 0.9 percent sodium chloride U.S.P., 0.04 percent formalin, 0.5 percent phenol as a preservative, and only trace amounts of beef heart extract, yeast extract, agar and hydrolysed derivatives of soya casein and agar. A difference between the composition of the military and civilian products has been resolved.

2. Labeling--a. Recommended use/indications. The vaccine is recommended for use in persons who have to be present in known plague endemic areas. The scheduled dose for adults is 1.0 ml, intramuscularly followed 3 months later by a dose of 0.2 ml intramuscularly. Proportionately smaller doses are specified for children aged 6 to 9 and for children aged 6 months to 5 years. Booster doses are recommended at 6 monthly intervals during residence in known plague endemic areas and consist of 0.2 ml intramuscularly. The standard precautions concerning the use of individual presterilized needles and syringes are included.

b. Contraindications. The labeling states that there are no real contraindications but advises not to give injections during upper respiratory infections.

3. Analysis--a. Efficacy--(1) Animal. This vaccine meets Federal requirements. Massive information is given concerning the immune response in rabbits and monkeys and the protection achieved in guinea pigs and mice.

Extensive data are available to show that the vaccine produces an antibody response in most recipients. Evidence that the vaccine was effective in protecting U.S. military personnel in Vietnam is provided in the work of Cavanaugh (Refs. 1 and 2).

b. Safety--(1) Animal. This vaccine meets Federal requirements.

(2) Human. Extensive clinical trials in man are cited in the submission to the Panel (Ref. 3) showing the occurrence of sore, swollen and red arms in a small percentage of subjects receiving their first injections, and in a far greater percentage receiving a full dose as a second injection (this is the reason that the recommended second dose is now 0.2 ml). An isolated reference is cited (Ref. 4) calling attention to the observation in 1 military clinic of 22 patients manifesting urticaria or other Type I allergic reactions after an injection of plague vaccine. The author describes skin tests on these subjects which support the belief that the reactions were due to the vaccine and not to constituents of the medium. He makes no attempt to even estimate the relative frequency of such reactions.

A great deal of additional data concerning reactions to this vaccine are available in the literature.

c. Benefit/risk ratio. In view of the data available which support the belief that the plague vaccine under consideration provides a significant degree of protection against plague, it is considered that the use of this vaccine in individuals who are liable to be exposed to plague is

entirely justified. Therefore the benefit-to-risk assessment of this product is satisfactory in those instances in which vaccine use is indicated.

4. Critique. The vial and package labels are clearly and explicitly marked. The package insert is on the whole much better than average; it is quite clearly written, however, Cutter Laboratories should provide a revised package insert based on civilian use.

5. Recommendations. The Panel recommends that this product be placed in Category I and that the appropriate license(s) be continued with the stipulation that labeling be revised in accordance with the recommendations of this Report.

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GENERIC STATEMENT

Typhoid Vaccine

Typhoid fever is a worldwide disease caused by the bacillus Salmonella typhi, which probably affects well over 1 million people a year. It consists of an infection starting in the lower small intestine but spreading to produce septicemia which, if not adequately treated, can cause many weeks of illness; the death rate, prior to antibiotic therapy, was 10 to 15 percent. Recently, strains that are resistant to antibiotics have appeared in several parts of the world, so that the risk of contracting a severe, prolonged illness if infected with Salmonella typhi is still present. Infection results from the consumption of food or water that has been contaminated directly or indirectly, by the excretions of a case or a carrier. The disease is uncommon in the United States but quite common in almost all countries with unsatisfactory sanitation.

Typhoid vaccine, is therefore widely used to protect travelers and others who may run a significant risk of contracting the infection.

Nature of Product

Typhoid vaccine consist of whole typhoid bacilli (Salmonella typhi), killed and preserved in any one of several ways. It is usually distributed as a suspension in saline or buffered saline at a concentration of 1 billion organisms per ml. One manufacturer supplies it - on military contract - as an acetone-killed and dried powder, together with a vial containing a suitable reconstituting fluid. The strain of Salmonella typhi used by all manufacturers is strain Ty 2.

The use of combined typhoid, paratyphoid A and B vaccine ("TAB" or "Triple typhoid vaccine") was discontinued in the United States because there is no evidence for the efficacy of the paratyphoid A component, and the paratyphoid B component was found to be effective only in much larger concentrations than were included in "TAB" vaccines.

## Production

The typhoid bacillus is usually grown for 24 hours at 35 to 37°C on veal infusion agar, and washed off with saline as a concentrated suspension. It is killed by heat, phenol, thimerosal or acetone, and resuspended at the indicated concentration, with either 0.5 percent phenol or 0.01 percent thimerosal added as a preservative. (The product for military use is prepared as noted earlier.) One manufacturer grows it in a semisynthetic medium in a fermenter. Some manufacturers centrifuge the crude harvest, discard the supernatant, and resuspend the sedimented bacteria in order to reduce the concentrations of reaction-producing soluble antigens and ingredients carried over from the medium.

The final vaccine is tested according to the United States standards. In addition to tests for sterility and safety, the vaccine must be tested for nitrogen content and potency. The latter is determined by a protection test in mice immunized with graded doses of vaccine and challenged with an intraperitoneal injection of a mucin suspension of a mouse virulent strain (Ty 2), compared against a United States standard vaccine preparation. The vaccine under test must have a potency of at least 0.6 times the standard.

## Use and Contraindications

The standard regimen for adults consists of 2 doses of 0.5 ml each subcutaneously at an interval of 3 to 4 weeks. Booster doses, when indicated, given at 3-year intervals, consist of 0.5 ml subcutaneously or 0.1 ml intradermally (acetone-killed vaccines are not recommended for intradermal injection because of the likelihood of excessive reactions). Proportionately reduced doses are recommended for children. Administration of the vaccine should be deferred in the presence of acute infections. It is generally believed that immunosuppressive agents may interfere with the effectiveness of the vaccine, although this is not well defined. Persons who have exhibited marked reactions to previous injections should be given reduced doses for booster injections.

## Safety

Inoculation with typhoid vaccine is frequently followed by local tenderness and swelling at the injection site, often accompained by mild to moderate fever generally lasting overnight but rarely more than 24 hours. Such reactions appear to be due, in primary immunization, to endotoxins, but there is clearcut evidence that untoward reactions--probably of the Arthus or delayed-sensitivity types--are especially common among individuals who have had repeated inoculations of typhoid vaccine. For this reason, booster injections should be given in smaller doses (0.1 ml) intradermally. In general, this procedure does appear to reduce the incidence and severity of untoward reactions; however, it has been found that acetone-killed and dried vaccines, for as yet unex-

plained reasons, cause a high incidence of severe local reactions with interdermal injections and hence this route is contraindicated with such vaccines.

Major reactions with permanent sequlae or death following typhoid vaccination are virtually unknown, and it is clear that--despite judicial allegations to the contrary--there is no evidence that bacterial endotoxins in the quantities present in bacterial vaccines can cause permanent sequlae. Moreover, the risk of excessive reactions is reduced by the mandatory ceiling on the nitrogen content of the vaccine. The vaccine must conform to the Bureau of Biologics' requirements for safety testing in animals.

## Efficacy

Until fairly recently, typhoid vaccines were prepared and used on a purely empirical basis. However, in recent years at least 10 well-controlled field trials have been carried out with various types of typhoid vaccine, in 5 different countries. It has been found that the efficacy of a particular vaccine varies considerably with the method of killing and the preservative added. Thus acetone-dried or formalin-killed whole cell vaccines have given up to 90 percent protection against "ordinary" exposure; heat-killed, phenol preserved vaccines gave somewhat less, or, if freeze-dried, considerably less protection. Alcohol-killed and preserved vaccines have given mediocre (30 to 50 percent) protection and chemical extracts and a vaccine prepared without H antigen have given little or no protection. (None of these last 3

classes of vaccine are in use within the United States.) It should be noted that studies in human volunteers indicate that against very large infectious doses of typhoid bacilli, even the best vaccines are ineffective.

As regards laboratory tests, the mouse protection test required by the Bureau of Biologics correlates with the field results in the case of acetone-killed and dried vaccines and also with freeze-dried heat killed phenol preserved vaccines. However, the mouse protection test correlates poorly with the results in man with alcohol-type vaccines. No such comparisons have been made with thimerosal-preserved vaccines.

The excellent field results with acetone-killed and dried vaccines were obtained with vaccines reconstituted just before use. However, the efficacy of such vaccines when distributed in the liquid state cannot be assumed to be identical.

Introduction of thimerosal as a preservative has not been tested by field trials. Nevertheless, laboratory tests show that thimerosal preservation is generally less deleterious than phenol and heat. The essentials concerning the various existing vaccines are shown in Table I.

It should be noted that no field trials have been carried out with typhoid vaccine prepared by any United States manufacturer. Nevertheless, the available typhoid vaccines are produced by methods similar to those employed for the production of vaccines that proved effective in field trials, or have introduced changes that could not, <u>a priori</u>, be

considered necessarily deleterious to the efficacy of the product. In spite of the uncertainties introduded by differing techniques of inactivation and preservation, the Panel considers that there is reasonable evidence of efficacy of available typhoid vaccines.

Special Problems

The major problem associated with typhoid vaccine is the lack of a laboratory test of potency which correlates consistently with field results with various vaccines in man. Furthermore, changes in preparation of the vaccine, even those that may be expected to be beneficial, create uncertainty in its evaluation. Meanwhile, however, it would be useful to study further the correlation of laboratory tests with human trials of formalin-preserved vaccine (see Table I).

This problem, however, can only be treated empirically until the mechanisms of immunity to typhoid fever are defined. Present knowledge indicates that immunity is not dependent on either H or Vi antigens alone, but that H antigen may be an essential component; however, it is possible that another, perhaps unidentified antigen, is also essential. It is not clear whether immunity is primarily humoral or cellular, systemic or local. If and when these questions are answered it should then be possible--in collaboration with studies in the field or in human volunteers--to identify a laboratory test that correlates satisfactorily with human protection. Related to the above, is the problem of preparing a less reactive vaccine.

TABLE I--INFORMATION ON CHARACTERISTICS OF CURRENT TYPHOID VACCINES

| Type | Effectiveness in field trials | Mouse protective potency | Antibody response in man H | O | Vi | Stability | Reactions |
|---|---|---|---|---|---|---|---|
| Heat killed, formalin preserved | +++ | ++ | + | + | + | ? | ? |
| Acetone-killed, kept in dry state | ++ to +++ | ++ | + | + | ++ | +++ | +++ (intradermal)<br>+ (s.c. or i.m.) |
| Heat-killed, phenol preserved | + to ++ | + | ++ | + | ± | ++ | + (any route) |
| Alcohol-killed and preserved | ± | ++ | + | + | ++ | ++ | ++ |
| Thimerosal killed and preserved | ? | ++ | + | + | + | ++ | + |
| Acetone-killed, thimerosal preserved | ? | ++ | + | + | + | variable | + |

± = borderline.
? = unknown.
+, ++, +++ = relative scale of response.*

*Because of variation in field and laboratory procedures only a relative scale is used in the table.

## Recommendations

1. Appropriate support should be given to studies aimed at clarifying the immune mechanism(s) in typhoid fever.

2. Field or volunteer studies designed to test promising vaccines or their fractions for protection against typhoid fever should be supported.

3. The search for laboratory tests which correlate well with results of vaccination in man should be continued.

## Basis of Classification

Proof of efficacy of typhoid vaccine is tied almost exclusively to field trials which are not feasible except in high endemic areas of the world. Classification of efficacy is therefore based upon production and preservation of vaccines known to be successful in such trials, and supported by a mouse protection test correlated with field results. Methods of inactivation and preservation of those vaccines that have not been previously subjected to field trials have been accepted by the Panel because on theoretical grounds there is no basis to believe that they would interfere with efficacy.

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SPECIFIC PRODUCT REVIEWS

TYPHOID VACCINE MANUFACTURED BY BUREAU OF LABORATORIES,

MICHIGAN DEPARTMENT OF PUBLIC HEALTH

1. Description. The vaccine is made from heat-killed Salmonella typhi (Ty 2 strain), suspended in phosphate buffered saline to a concentration of not more than 1,000 x $10^6$ cells per ml. The material prepared since 1969 is preserved with 0.01 percent thimerosal. The vaccine contains 8 protective units per ml.

2. Labeling--a. Recommended use/indications. The labeling follows the Public Health Services Advisory Committee on Immunization Practices recommendations and is indicated for intimate contacts with known cases of typhoid fever or carriers; for medical or hospital personnel; and for individuals contemplating travel to endemic areas.

b. Contraindications. (1) Acute respiratory disease; (2) in children with histories of febrile convulsions or cerebral damage; and (3) patients on corticosteroid and/or immunosuppressive drugs - since the immune response may be suppressed.

3. Analysis--a. Efficacy--(1) Animal. This product meets Federal requirements.

(2) Human. No field trials have been performed with this product.

b. Safety--(1) Animal. This product meets Federal requirements.

(2) Human. The manufacturer reports that no complaints have been received in the 10 year period from 1961 to 1972 during which many hundred thousand doses were distributed. Local reactions occured with intradermal injections in all (27/27 adults) with a past history of typhoid vaccine (2.43 cm to 6.5 x 7 cm erythema).

c. Benefit/risk ratio. The benefit-to-risk assessment of this product cannot be determined with certainty because there is no supporting field trial evidence of efficacy for this specific product. However, it is likely that the benefit-to-risk assessment of this product is satisfactory. (See Generic Statement.)

4. Critique. Although this vaccine should meet required standards of preparation (it is heat-killed and was phenol preserved), since 1969 it has been preserved with thimerosal. The latter is presumed to be at least as desirable a method of preservation as is phenol. (See generic statement.)

5. Recommendations. The Panel recommends that this product be placed in Category I and that the appropriate license(s) be continued with the stipulation that labeling be revised in accordance with the recommendations of this Report.

TYPHOID VACCINE MANUFACTURED BY ELI LILLY AND COMPANY

1. Description. This typhoid vaccine is a suspension of the Ty 2 strain of Salmonella typhi grown in a semisynthetic liquid medium. The organisms are killed by acetone which is then removed. The organisms are resuspended in buffered physiological saline, containing 0.01 percent thimerosal as preservative. The final vaccine contains no more than 1,000 million typhoid organisms per ml, no more than 0.023 mg of nitrogen per ml. The final product is standardized to 8 protective units per ml.

2. Labeling--a. Recommended use/indications. This product is recommended for active immunization against typhoid fever under the following circumstances: (1) Intimate exposure to a known carrier; (2) community or institutional outbreaks; and (3) foreign travel to endemic areas. The label cautions against intradermal administration.

These recommendations agree fully with those of the Public Health Service Advisory Committee on Immunization Practices, as does the recommended schedule for dosage and administration.

b. Contraindications. It is recommended that vaccination be avoided during an acute illness. The labeling further contains a caution about the administration of typhoid vaccine during chronic steroid therapy, implying that steroid therapy may so modify host defense mechanisms that an otherwise effective vaccine may be rendered ineffective.

3. Analysis--a. Efficacy--(1) Animal. This product meets Federal requirements.

(2) Human. In a study carried out by Eli Lilly and Company (Ref. 1) when a change to acetone inactivation of the vaccine was made, 60 adult males were randomly divided into 3 groups, 2 receiving separate lots of acetone-killed vaccine, and 1 receiving Eli Lilly and Company's heat-phenol inactivated vaccine. Each received two 0.5 ml doses 4 weeks apart, and some received a third dose 4 weeks later. They were observed for 48 hours after each dose. No significant differences were noted among vaccinees in height of H, O, or Vi antibody titer according to vaccine used. The actual data, however, are not provided.

The general body of data supporting the efficacy of acetone-killed vaccines is cited in the manufacturer's submission (Ref. 1), but Eli Lilly and Company's vaccine per se was not used.

b. Safety--(1) Animal. This product meets Federal requirements.

(2) Human. In the study cited above, there was no difference in local reactivity among recipients of the 3 vaccines, although the absolute numbers were not cited. Six of the subjects complained of constitutional reactions including chills or malaise during the 48 hour observation period, but all remained afebrile, and the complaints came equally from all 3 vaccine groups. There were no allergic reactions.

The manufacturer's marketing experience indicates that a few million doses of the vaccine were distributed in the 5 year period 1968 to 1972, and that 18 complaints were received of local or systemic reactions.

c. Benefit/risk ratio. The benefit-to-risk assessment of this product cannot be determined with certainty owing to the lack of supporting field trail evidence of efficacy of acetone-killed vaccines

preserved in the liquid state with thimerosal. However, it is likely that the benefit-to-risk assessment of this product is satisfactory. (See Generic Statement.)

4. Critique. This vaccine is killed by acetone but its preservation by thimerosal introduces a variable which has not yet been tested by field trial. However, animal studies and theoretical considerations strongly suggest that this vaccine should be effective in field trials. The latter may not be feasible with this product in the foreseeable future.

The labeling should be revised to reflect more current knowledge of the effect of corticosteroid therapy on immunoglobulin synthesis, particularly with regard to the dose and duration of steroid therapy. In addition, references to the need for "separate heat-sterilized syringe and needle" are quite dated, and should be revised to reflect contemporary practice as well as contemporary knowledge of hepatitis B.

5. Recommendations. The Panel recommends that this product be placed in Category I and that the appropriate license(s) be continued with the stipulation that labeling be revised in accordance with the recommendations of this Report.

TYPHOID VACCINE MANUFACTURED BY MASSACHUSETTS PUBLIC HEALTH BIOLOGIC LABORATORIES

1. Description. The final vaccine contains no more than one thousand million bacterial cells per ml (strain Ty 2) suspended in phosphate buffered saline containing 0.01 percent thimerosal. The bacilli are killed by thimerosal at room temperature, but no further details of the manufacturing process are given.

2. Labeling--a. Recommended use/indications. For persons for whom immunization against typhoid fever is indicated. The indications are not specified, but reference is made to the Public Health Services Advisory Committee on Immunization Practices recommendations. For primary immunization 2 doses of 0.5 ml subcutaneously on 2 occasions, separated by 4 or more weeks are given to adults and children over 10 years of age. For children 6 months to 10 years the procedure is the same except that the dose is 0.25 ml.

Under conditions of continued or repeated exposure a single booster dose should be given at least every 3 years.

Boosters can also be given with an intradermal dose of 0.1 ml, which generally would give less reaction.

b. Contraindications. None are mentioned, although a warning is given to review the history of the patient regarding possible sensitivity to the product.

3. Analysis--a. Efficacy--(1) Animal. This product meets Federal requirements and exceeds the potency of an analagous heat-killed, phenol preserved vaccine in the mouse protection test.

(2) Human. No information was provided on this particular product.

b. Safety--(1) Animal. This product meets Federal requirements.

(2) Human. No controlled, partially controlled or uncontrolled studies have been carried out by the Massachusetts Public Health Biologic Laboratories. No fatal reaction following administration of typhoid vaccine has been documented by the Massachusetts Public Health Biologic Laboratories. However, it is well known that there may be many local reactions and some general reactions in adults following administration of the vaccine. No data from the complaint file are given.

c. Benefit/risk ratio. Assuming the product is effective, and the person to be vaccinated is at some risk of acquiring typhoid fever, the benefit-to-risk assessment should be satisfactory. (See Generic Statement.)

4. Critique. No clinical tests have been carried out on this particular product, but data from unpublished mouse protection tests suggest that the manufacturing process yields a vaccine equal or superior to vaccines of proven efficacy (See Generic Statement). The label is vague on indications for use.

5. Recommendations. The Panel recommends that this product be placed in Category I and that the appropriate license(s) be continued with the stipulation that labeling be revised in accordance with the recommendations of this Report.

TYPHOID VACCINE MANUFACTURED BY MERCK SHARP & DOHME,

DIVISION OF MERCK & CO., INC.

1. Description. The brief submission by Merck Sharp & Dohme represents a phenol-inactivated typhoid vaccine. The appropriate strain of typhoid bacilli is used and the final concentration is 1 billion organisms per ml. It is diluted in a buffered solution of physiologic sodium chloride. The preservative is phenol, 0.5 percent. The bacteria are inactivated by phenol, apparently without heat. No other information is given regarding its production.

2. Labeling--a. Recommended use/indications. The package insert, now 11 years old, recommends a dosage schedule at variance with current recommendations. The description of the method of preparation is outdated.

b. Contraindications. The labeling statement is acceptable.

3. Analysis--a. Efficacy--(1) Animal. This product met Federal requirements when it was produced. No other information is supplied.

(2) Human. The only information provided is related to studies of generic typhoid vaccines.

b. Safety--(1) Animal. The manufacturer's submission states that the product meets Federal requirements.

(2) Human. No data are provided.

c. Benefit/risk ratio. The benefit-to-risk assessment of this product cannot be determined.

4. Critique. This is a typhoid vaccine, apparently phenol inactivated, which appears to meet United States standards for animal safety.

No other information regarding its efficacy or safety is provided. The labeling is outdated.

5. Recommendations. The Panel recommends that this product be placed in Category IIIC and that the appropriate license be revoked for administrative reasons because this product is not marketed and there are insufficient data on labeling, safety, and effectiveness.

TYPHOID VACCINE MANUFACTURED BY TEXAS DEPARTMENT OF HEALTH RESOURCES

1. Description. This product contains approximately 1,000 million organisms of Salmonella typhi per ml, strain Ty 2, killed by heat and phenol. Diluent is 0.02 M phosphate buffered saline, pH 7.2 to 7.3; 1:10,000 thimerosal is added. Each milliliter of vaccine contains 8 potency units in accordance with the United States standard typhoid vaccine.

2. Labeling--a. Recommended use/indications. Routine immunization is not recommended in the United States. Selective immunization is, however, indicated in the following situations: (1) Intimate exposure to a known typhoid carrier as would occur with continued household contact; (2) community or institutional outbreaks of typhoid fever; and (3) foreign travel to areas where typhoid fever is endemic.

Primary immunization; dosage and schedule: (a) Adults and children over 10 years of age; 0.5 ml subcutaneously on 2 occasions, separated by 4 or more weeks; and (b) children 6 months to 10 years of age; 0.25 ml subcutaneously on 2 occasions, separated by 4 or more weeks.

Booster doses should be given at least every 3 years under conditions of continued or repeated exposure to typhoid as follows: Adults and children over 10 years of age, 0.5 ml subcutaneously or 0.1 ml intradermally; and children 6 months to 10 years of age, 0.25 ml subcutaneously or 0.1 ml intradermally.

b. Contraindications. Immunization of persons with acute febrile illness or other active infection should be deferred.

3. Analysis--a. Efficacy--(1) Animal. This product meets Federal requirements.

(2) Human. No information from studies conducted on this particular product.

b. Safety--(1) Animal. This product meets Federal requirements.

(2) Human. No controlled studies are presented. Over the past 10 years several million doses of the vaccine have been distributed in Texas without reports of serious reactions.

c. Benefit/risk ratio. Assuming the product is effective, the benefit-to-risk assessment should be satisfactory. (See Generic Statement.)

4. Critique. The vaccine is killed and preserved by heat and phenol. In addition, thimerosal is added as a preservative. The latter should not affect the vaccine adversely although field trials have not yet confirmed this assumption. However, such field trials with this vaccine are not feasible in the foreseeable future.

5. Recommendations. The Panel recommends that this product be placed in Category I and that the appropriate license(s) be continued with the stipulation that labeling be revised in accordance with the recommendations of this Report.

TYPHOID VACCINE (ACETONE INACTIVATED) MANUFACTURED BY WYETH LABORATORIES, INC.

1. Description. This typhoid vaccine contains 1 billion acetone killed Salmonella typhi (Ty 2 organisms) per ml. The organisms are inactivated by precipitation with acetone and warming at 37° C for 24 hours. The vaccine is distributed in dried form with a sterile diluent containing 0.5 percent phenol as a preservative for reconstitution.

2. Labeling--a. Recommended use/indications. For primary immunization for adults and children of 10 years of age and older, 2 doses of 0.5 ml each, injected subcutaneously or intramuscularly, are recommended with an interval of 4 or more weeks. For children 6 months through 9 years of age the subcutaneous or intramuscular injection of 2 doses of 0.25 ml each is recommended at an interval of 4 or more weeks. For reinforcement of immunity for adults and children of 10 years of age and older, 0.5 ml injected subcutaneously or intramuscularly is recommended. For children 6 months through 9 years of age the dose for reinforcement is 0.25 ml, injected subcutaneously or intramuscularly. The timing of reinforcement doses is not specified, but instead reference is made to military recommendations, inasmuch as this product is used primarily by the Armed Forces. Intradermal innoculation is contraindicated.

b. Contraindications. The manufacturer recommends deferral of immunization in the presence of an acute respiratory or other active infection.

3. Analysis--a. Efficacy--(1) Animal. This product meets Federal requirements.

(2) Human. Field trials conducted by the World Health Organization employing vaccines very similar to this product have displayed a high degree of efficacy.

b. Safety--(1) Animal. This product meets Federal requirements.

(2) Human. Typhoid vaccines in general produce high rates of local reactions and some systemic reactions, neither of which are serious. Severe reactions are very rare. This preparation appears to yield reactions at rates no greater than those expected.

c. Benefit/risk ratio. The benefit-to-risk assessment of this vaccine is satisfactory when compared with typhoid vaccines in general. (See Generic Statement.)

4. Critique. This is one of the few available typhoid vaccines which has been prepared by methods virtually identical to those vaccines which were most efficacious in field trials. Its efficacy is therefore well established.

5. Recommendations. The Panel recommends that this product be placed in Category I and that the license(s) be continued with the stipulation that labeling be revised in accord with the recommendations of this Report.

TYPHOID VACCINE (HEAT-PHENOL INACTIVATED) MANUFACTURED BY WYETH LABORATORIES, INC.

1. Description. The typhoid vaccine contains 1 billion Salmonella typhi (Ty 2 strain) heat-phenol killed organisms per ml. The organisms are killed by suspending them in sodium chloride, heating to 56° C for 1 hour, and then adding 0.5 percent phenol and maintaining the batch at room temperature thereafter for 4 days. Phenol 0.5 percent is added as a preservative in the final diluent.

2. Labeling--a. Recommended use/indications. For primary active immunization of adults and children greater than 10 years of age, 2 doses of 0.5 ml each subcutaneously are recommended at an interval of 4 or more weeks. For children of 6 months to 10 years of age, 2 subcutaneous doses of 0.25 ml are recommended with an interval of 4 or more weeks. When necessary to complete immunization in a shorter period of time, the manufacturer recommends the above doses administered subcutaneously on 3 occasions at weekly intervals.

If necessary to maintain immunity, the manufacturer recommends a reinforcing dose at least every 3 years. However, if an interval of more than 3 years has elapsed since the last dose, a single reinforcing dose is satisfactory. Reinforcing doses for adults and children over 10 years of age comprise either 0.5 ml subcutaneously or 0.1 ml intracutaneously. For children 6 months to 10 years of age, 0.25 ml subcutaneously or 0.1 ml intracutaneously is recommended.

b. Contraindications. The manufacturer recommends deferral of immunization in the presence of an acute respiratory or other active infection.

3. Analysis--a. Efficacy--(1) Animal. This product meets Federal requirements.

(2) Human. Field trials conducted by the World Health Organization employing vaccines very similar to this product have displayed efficacy.

b. Safety--(1) Animal. This product meets Federal requirements.

(2) Human. Typhoid vaccines in general produce high rates of local reactions and some systemic reactions, neither of which are serious. Severe reactions are very rare. This preparation appears to yield reactions at rates no greater than those expected.

c. Benefit/risk ratio. The benefit-to-risk assessment of this vaccine is satisfactory when compared with typhoid vaccines in general. (See Generic Statement.)

4. Critique. This heat phenol inactivated typhoid vaccine is analogous to those found effective by field trials (see Table I) and would therefore appear to be efficacious.

5. Recommendations. The Panel recommends that this product be placed in Category I and that the license(s) be continued with the stipulation that labeling be revised in accord with the recommendations of this Report.

REFERENCES

(1) BER VOLUME 2050.

## PASSIVE IMMUNIZATION PRODUCTS

### Generic Statement on Botulinus Antitoxin

Botulism is a paralytic disease caused by the action of a protein neurotoxin elaborated by Clostridium botulinum. Clostridium botulinum, a spore-forming organism closely related to Clostridium tetani, is widely distributed in nature and can regularly be found in soils and from marine sources. Six types of Clostridium botulinum (A-F) are recognized; each produces an immunologically distinct neurotoxin. These are among the most powerful toxins known; one microgram contains 200,000 minimal lethal doses for a mouse, and is very close to the lethal dose for man.

The disease usually results from the ingestion of uncooked food of animal origin, e.g., sausage, spiced meat, or smoked fish, or improperly canned fruits or vegetables, in which spores of the organism contaminated the product, germinated and produced toxin. Food that is not obviously spoiled may still contain botulinus toxin. Thus, the disease is usually not an infection, but rather an intoxication. However, occasional cases of botulism result from infection of a surgical or traumatic wound with Clostridium botulinum, followed by toxin production in vivo. There is also strong suggestion that some cases of botulism result from toxin formation by Clostridium botulinum organisms in the human gastrointestinal tract.

Most human botulism is caused by types A, B and E. Botulism caused by improperly canned vegetables or improperly preserved meat

products is generally due to types A or B; most of the type E botulism reported in the United States has been traced to fish or fish products. Only two outbreaks of type F botulism have been reported. Types C and D produce disease almost exclusively in animals.

Although the spores are relatively heat resistant, requiring pressure sterilization to insure killing, botulinus toxin is relatively heat-labile, being completely inactivated by a temperature of 100° C for 10 minutes.

The disease is rare, but often fatal. From 1910 to 1919, 246 cases were reported in the United States. A series of studies by K. F. Meyer and his associates in the early 1920's defined the epidemiology of botulism, the foods most often incriminated, and the conditions necessary for the destruction of Clostridium botulinum spores. These studies led to strict controls on the commerical canning industry, and most cases of botulism in the last 25 years have followed consumption of improperly canned, home preserved foods. From 1970 to 1973, 30 outbreaks of foodborne botulism, involving 91 cases and 21 deaths were reported to the Center for Disease Control. Six cases of wound botulism were reported during the same period. Very recently, investigators in California have described a syndrome of infant botulism; the epidemiology and pathogenesis of botulism in children less than one year of age is currently under active investigation.

Treatment of botulism is directed toward 3 major goals. First, unadsorbed toxin should be removed from the gastrointestional tract. This can be accomplished by an emetic if the suspected food was recently

ingested, or more commonly by purging and enemas. Second, circulating neurotoxin can be neutralized by the administration of antitoxin. It is unlikely that antitoxin has any neutralizing effect on toxin already fixed to nerve tissue. Finally, assisted respiration is used to compensate for the neuromuscular blockade and to tide the patient over the period of respiratory paralysis.

## Nature of Product

Botulism antitoxin trivalent, types A, B and E, and botulism antitoxin, type E, consist of the partially purified globulin fraction from the serum of horses hyperimmunized with multiple sequential doses of botulism toxoid.

## Production

Botulism antitoxin, types A, B and E are generally produced in the same animal by immunizing horses with subcutaneous injections of alum-precipitated formalinized toxoids prepared from Clostridium botulinum, types A, B and E. To produce monovalent type E botulism antitoxin, only the type E toxoid is used for immunization. Hyperimmunization is begun with subcutaneous injections of gradually increasing amounts of the liquid toxoid at weekly intervals. Trial bleedings are taken periodically, and when antitoxin titers are sufficiently high, the serum is harvested by plasmapheresis. The plasma is pooled, defibrinated, subjected to pepsin digestion followed by ammonium sulfate fractionation, dialyzed and adjusted to yield approximately a 20 percent concentration of serum proteins. An average of 50 percent of the antitoxin activity originally present in the plasma is recovered in the final concentrate.

The digested, fractionated, dialyzed product is adjusted to a concentration suitable for filling, and tested for identity, safety and potency in units per ml in toxin-antitoxin neutralization tests in graded dilutions in groups of mice. Phenol is added as a preservative to a concentration of 0.45 percent w/v, and the product is filled with a 20 percent excess or more, according to Federal standards related to the stated expiration date.

Recommended use/indications

Evidence concerning the exact amount of circulating antitoxin needed to neutralize experimental botulinus toxin poisoning is incomplete. Animal evidence suggests that the outcome of treatment depends largely on the time interval elapsing after the onset of symptoms, and before the peak of circulating administered antitoxin is reached. Therefore it is strongly recommended that patients should be treated promptly with botulism antitoxin trivalent types A, B and E, as soon as the clinical diagnosis of botulism is suspected. Prior to the injection of this material, if circumstances permit, the patient should be questioned regarding any history suggesting sensitivity to horses or horse serum, and should be tested for such sensitivity by conjunctival (1:10 dilution) or intradermal (1:100 dilution) tests with the serum for freedom from reactions. Suitable test kits for this purpose are sometimes available. Some experts advocate instead a tolerance test with 0.1 ml of a 1:100 dilution given subcutaneously. No test system is totally reliable, and the patient must be watched for at least 1 hour after the antitoxin has been injected.

Best results in the treatment of botulism are likely to be obtained if large doses of antitoxin are given early in the disease, the object being to provide an excess of circulating antitoxin as early as possible. In order to insure the most rapid neutralization of all toxin in the tissue and fluids, most authorities recommend prompt intravenous administration of 1 vial (7,500 international units of type A, 5,500 international units of type B and 8,500 international units of type E), injected very slowly at a dilution of 1:10, the solution to be at ambient temperature before being injected.

In order to provide a reservoir of antitoxin for subsequent adsorption, an additional equal dose may be given by intramuscular injection. Further doses are indicated in 2 to 4 hours if the signs and symptoms worsen. Because antitoxin remains in the circulation for over 30 days, the recommended dose should be given immediately, rather than in multiple small doses administered over a long period.

The recommended prophylactic dose for an individual who has eaten food suspected of being infected with Clostridium botulinum is 1,500 to 7,500 international units of type A, 1,100 to 5,500 international units of type B, and 1,600 to 8,500 international units of type E given intramuscularly, depending on the amount of food eaten. If signs or symptoms of botulism appear, further treatment should be initiated with intravenous antitoxin.

Unless there is unequivocal evidence that the disease under treatment or preventive therapy is type E botulism, the trivalent antitoxin

(types A, B and E), is always recommended. If the disease is known to be type E botulism, therapy with monovalent type E antitoxin is justified. Individuals who exhibit apparent sensitivity to horse serum should nevertheless receive antitoxin, employing recommended schedules for gradual desensitization with increasing doses of antitoxin administered over several hours until the total dose has been given.

## Safety

Federal regulations specify that botulism antitoxin be tested to insure sterility, and contain an appropriate preservative in specified amounts. The product must meet prescribed test results for freedom from pyrogenicity in animals.

The most significant problems regarding the safety of botulism antitoxin relate to sensitivity to horse serum. Two types of hypersensitivity reactions occur: anaphylaxis and serum sickness. These reactions cannot always be predicted in advance by sensitivity testing, and may not be prevented by desensitization.

Anaphylactic reactions to horse serum, fortunately the less common of the two, can occur without any known prior sensitization. They occur immediately or within a few minutes following injection, and are manifest by severe respiratory distress, collapse and shock. Even with prompt administration of epinephrine, death may occur in 10 percent or more of cases.

Serum sickness following horse serum occurs 6 to 21 days after an individuals' first injection. Prior sensitization is not required, although previous injections increase the likelihood of serum sickness and decrease the latent period between injection and onset of symptoms to as little as a few hours. The larger the dose of serum, the more likely is serum sickness to occur. Rates of serum sickness following horse serum vary, but range from 2 to 30 percent, and are directly dose dependent. In the most recent United States experience, however, only 7 percent of recipients of botulism antitoxin developed serum sickness. The overall rate of adverse reactions reported to the Center for Disease Control was 21 percent.

## Efficacy

There is limited evidence that type E antitoxin is effective in preventing death in man when given after the onset of symptoms, but there is little data on the efficacy of types A and B in man. In animals, type E and type A antitoxins appear to be effective, but the efficacy of type B antitoxin has never been conclusively demonstrated.

Almost all of the human botulism outbreaks in Japan have been due to type E. In 20 outbreaks before antitoxin was used, the mortality rate was 28 percent; in 15 outbreaks after the use of type E antitoxin began, the mortality rate was reduced to 4 percent. The 2 groups may not have been comparable in other respects such as quality of supportive therapy or the duration of symptoms prior to treatment. In the more recent reports of type E botulism in the United States, as reported by

Koenig and Whittaker, 5 of 7 patients not given type E antitoxin died, but none of 8 patients given type E antitoxin died. Again, the treated and untreated patients may not have been comparable in other respects. There is thus evidence, albeit uncontrolled, of the effectiveness of botulism antitoxin in man but only for type E.

Despite the lack of convincing evidence as to the efficacy of types A and B botulism antitoxin, the advisability of its use is firmly established in medical practice, and will presumably continue so unless chemical means are devised to circumvent the neuroparalytic effects of botulinus toxin.

## Special Problems

Botulism is fortunately a rare disease in the United States. The number of cases reported in the past 10 years has varied annually from a low of 5 to a high of 34.

Since one consequence of rising food prices may well be an increase in home canning, education of the home canner and consumer is the most pressing need in the prevention and control of botulism. Public health agencies should provide information to the home canner about proper techniques and common errors involved in the preservation of foods.

The recent increase in contaminated commercial products suggest that new Federal regulations for canning low-acid foods are crucial to the prevention of processing errors by the canning industry. This should be a joint responsibility of the Center for Disease Control, the Food and Drug Administration and the Department of Agriculture. Control

measures developed and initiated by the smoked fish industry after 3 outbreaks involving smoked fish in the mid-1960's serve as a model for responsible action by the food industry.

Botulinus toxoid is available to permit development of botulism immune globulin of human origin. With so few cases occurring every year, this has understandably been given a rather low priority in research and development of biological products.

Several reports have appeared since 1967 describing the use of guanidine hydrochloride in the treatment of botulism. The drug is thought to act by enhancing the release of acetylcholine from nerve terminals. Reported cases have generally shown improvement with oral doses of 35 to 50 mg per kg per day, and in some instances the beneficial effect of the drug has been documented with neurophysiologic studies. Nevertheless, these studies have not been controlled, and the efficacy of guanidine hydrochloride and other drugs that act at the myoneural junction remains in question. For these reasons the use of these drugs should not preempt the administration of botulism antitoxin.

## Recommendations

1. Encourage educational programs directed at the home canner; 2. encourage the enforcement of Federal regulations established for the canning of low acid foods and other high risk foods in the commercial industry; 3. consideration should be given to the development of botulism immune globulin of human origin; and 4. support studies designed to elucidate the mechanism of action of botulinus toxin, and the development of pharmacologic agents that circumvent or minimize the neuroparalytic effects of the toxin.

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(5) Merson, M. H. and V. R. Dowell, "Epidemiologic, Clinical and Laboratory Aspects of Wound Botulism, New England Journal of Medicine, 289:1005, 1973.

(6) Koenig, M. G., D. J. Drutz, A. I. Mushlin, W. Schaffner and D. E. Rogers, "Type B Botulism in Man," American Journal of Medicine, 42:208, 1967.

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(8) Arnon, S. S., T. F. Midura, S. A. Clay, R. M. Wood and J. Chin, "Infant Botulism: Epidemiological, Clinical and Laboratory Aspects," Journal of the American Medical Association, 237: 1946, 1977.

SPECIFIC PRODUCT REVIEWS

BOTULISM ANTITOXIN, TYPES A, B AND E AND BOTULISM ANTITOXIN, TYPE E MANUFACTURED BY CONNAUGHT LABORATORIES LIMITED

1. Description. Botulism antitoxin, types A, B and E, and monovalent type E, as supplied by Connaught Laboratories, is a refined and concentrated preparation of globulins modified by enzymatic digestion. The product is obtained from horses immunized with botulism toxoids, types A, B and E, or type E alone. The product is purified and concentrated by ammonium sulfate precipitation, pepsin digestion, and ultrafiltration. Phenol is added as a preservative at a concentration of 0.45 percent w/v.

Extensive details of the manufacturing process are provided. The trivalent product contains 7,500 international units type A antitoxin, 5,500 international units type B antitoxin, and 8,500 international units type E antitoxin per vial (10 ml). The monovalent product contains 5,000 international units type E antitoxin per 2 ml vial.

2. Labeling--a. Recommended use/indications. For the prevention and/or treatment of botulism.

b. Contraindications. There are extensive precautionary statements about testing for sensitivity to horse serum, but no absolute contraindications are specified.

3. Analysis--a. Efficacy--(1) Animal. This product meets Federal requirements. A toxin-antitoxin neutralization test is carried out in mice for each individual component of the trivalent antiserum, in order to determine the unitage.

(2) Human. No specific data are cited, but frequent references are made to the work of Dolman, in Vancouver. A statement is made in the submission, (Ref. 1) as follows:

> "To date our botulism antitoxin is used in Canada and is stocked by the National Communicable Disease Center, Atlanta, Georgia. From their reports in Morbidity and Mortality we can assume that when the antitoxin is administered the effect is life-saving in most cases."

Such an assumption is unjustified. However, the report of the Tennessee epidemic (See Generic Review), not cited in the manufacturer's submission, demonstrated the efficacy of type E antitoxin.

b. Safety--(1) Animal. This product meets Federal requirements.

(2) Human. According to the Center for Disease Control's surveillance of reactions to botulism antitoxin, a 17 percent frequency of reactions to this product is mentioned in the Morbidity and Mortality Weekly Report.

c. Benefit/risk ratio. The benefit-to-risk assessment of this product is satisfactory.

4. Critique. The labeling is clear and adequate.

5. Recommendations. The Panel recommends that this product be placed in Category I and that the appropriate license(s) be continued.

BOTULISM ANTITOXIN MANUFACTURED BY LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO.

No data have been provided by the manufacturer for botulism antitoxin, for which they are presently licensed. In the absence of aany information from the manufacturer, the Panel can make no determination regarding the relative benefits and risks of this product.

Recommendation. The Panel recommends that this product be placed in Category IIIC and that the appropriate license be revoked for administrative reasons because this product is not marketed and there are insufficient data on labeling, safety, and effectiveness.

REFERENCES

(1) BER VOLUME 2061.

GENERIC STATEMENT

Diphtheria Antitoxin

Diphtheria is an infectious and communicable disease of man which usually involves the upper respiratory tract and sometimes produces skin infections. The causative agent is Corynebacterium diphtheria, a gram-positive bacillus with metachromatic granules. Upper respiratory diphtheria is characteristically associated with the production of a pseudomembrane in the nasal passage, pharynx, and/or larynx, and with the appearance of systemic symptoms due to absorption of an exotoxin. Fifty years ago there were approximately 200 cases per 100,000 population in the United States each year (roughly 200,000 cases annually). This has decreased to a rate of about 0.1 per 100,000 population in recent years (200 to 400 cases annually). Approximately 10 percent of patients with diphtheria succumb. Death may be due to respiratory obstruction by the membrane or to remote effects of the toxin upon the myocardium or peripheral nervous system.

Because the morbidity and mortality of diptheria are largely a consequence of the toxin elaborated by the organism, antiserum (antitoxin) prepared by immunizing horses has been used for nearly 80 years in the treatment of the disease and for its prevention in exposed, susceptible individuals. This approach to control of the disease is only partially successful, because the disease is already well established by the time it is recognized, and toxin that has been absorbed and fixed to cells is unaffected by antitoxin.

Further, antitoxin does nothing to prevent spread of the toxigenic causative organism. Penicillin or other effective antibiotic agents will usually eradicate the organism but, because they have no effect against toxin, antibiotics are only an adjunct to therapy of clinical diphtheria.

Since neither passive immunization with antitoxin nor therapy with antimicrobial agents provides an entirely satisfactory approach to the control of diphtheria, active immunization of humans against the toxin is the safest, most effective control measure. The reduction in morbidity and mortality from diphtheria in the United States during the past half century is largely attributable to widespread immunization against the toxin. But because significant segments of the United States population have not received adequate active immunization against diphtheria employing the toxoid, between 200 and 400 cases of diphtheria continue to occur yearly. For these individuals therapy with antitoxin is required.

## Description

Diphtheria antitoxin is a preparation of hyperimmune serum prepared in horses immunized against diphtheria toxin.

## Production

Diphtheria antitoxin is prepared by hyperimmunizing horses with diphtheria toxoid and diphtheria toxin until high levels of serum antitoxin activity are achieved. The horses must be demonstrated to be free of communicable disease.

Plasma containing satisfactory titers of antitoxin is concentrated by precipitation and dialysis and usually partially refined by pepsin digestion. Final concentration of antitoxin is at least 500 units per ml. Sterilization is achieved by microfiltration and an appropriate preservative is added. Each lot must meet requirements for sterility and freedom from pyrogenicity according to Federal regulations. Potency is tested by comparison with United States standard antitoxin.

Use and Contraindications

The major use of diphtheria antitoxin is for the treatment of clinical diphtheria. Treatment should be initiated immediately, prior to definitive bacteriologic diagnosis, in individuals in whom there is reasonable clinical suspicion of diphtheria. Delay in administration is to be avoided, because the antitoxin only neutralizes circulating toxin; toxin already fixed to tissue is unaffected. Delay allows increasing amounts of toxin to bind to tissue and is associated with a progressive increase in case fatality.

The dose of antitoxin recommended by most authorities is between 20,000 and 80,000 units, depending on the size of the patient, the severity and duration of infection. The entire dose should be given at one time; some authorities recommended that up to one-half be given intravenously, and the rest intramuscularly. Because sensitivity to horse serum is frequent in humans, sensitivity testing and a carefully taken history of any findings suggesting sensitivity to horses, horse dander or horse serum are mandatory. Tests should be performed by both

intradermal and conjuctival routes, with extreme precautions in case of any adverse reactions. Individuals with diphtheria exhibiting apparent sensitivity to horse serum nevertheless should receive antitoxin, employing recommended schedules for gradual "desensitization" with increasing doses of antitoxin administered over several hours until the total dose has been given.

Important adjuncts to therapy include general supportive measures, maintenance of the airway in patients with laryngeal diphtheria (diphtheritic croup), and administration of antimicrobial drugs active against Corynebacterium diphtheriae (erythromycin, lincomycin, penicillin, rifampin). Antimicrobial drugs, however, are only adjuncts to therapy and must not be used instead of antitoxin.

For the prevention of diphtheria in exposed, susceptible individuals (persons who are Schick test positive and/or who have not been immunized), diphtheria antitoxin, 1,000 to 5,000 units administered intramuscularly may be used subsequent to testing for sensitivity to horse serum (see Special Problems).

There are no absolute contraindications to the use of diphtheria antitoxin in the presence of diphtheria.

## Safety

Federal regulations specify that diphtheria antitoxin must be tested to ensure sterility, and contain an appropriate preservative in specified amount. The product must meet prescribed tests for freedom from pyrogenicity.

The most significant problems regarding the safety of diphtheria antitoxin relate to sensitivity to horse serum. Two types of hypersensitivity reactions occur: anaphylaxis and serum sickness. These reactions cannot always be predicted in advance by sensitivity testing, and may not be prevented by "desensitization."

Anaphylactic reactions to horse serum, fortunately the less common of the two, can occur without any known prior sensitization of any identifiable sort. They occur immediately or within a few minutes following injection and most characteristically comprise collapse and shock. Even with prompt administration of epinephrine, death may occur in 10 percent or more cases.

Serum sickness following horse serum occurs 6 to 21 days after an individual's first injection. Prior sensitization is not required, although previous injections increase the likelihood of serum sickness and decrease the latent period between injection and onset of symptoms to as little as a few hours. The larger the dose of serum, the more likely is serum sickness to occur. The major manifestations of serum sickness are fever, arthritis, lymphadenopathy and urticaria. Fatalities are rare except in instances of laryngeal edema. Symptoms persist for days or weeks. Rates of serum sickness following horse serum vary and are directly dependent on the dose. Indeed, the administration of 100 ml produces serum sickness in 90 percent of recipients.

## Efficacy

The degree of effectiveness of diphtheria antitoxin in the therapy of diphtheria is not precisely established. Although many studies are reported, most are beset with problems of study design sufficient to cause concern about the exactitude of the results. For example, a number of studies indicate that individuals who received antitoxin in the first day or two of the illness exhibited fewer complications and increased survival compared to those receiving treatment later, but there are questions about the comparability of cases treated early and late. However, in the early experience, when supplies of antitoxin were erratic, the contrast between patients treated with it and those unable to be so treated was reported as very striking. Further, there appear to be secular changes in the severity and incidence of diphtheria, negating comparisons from year-to-year and decade-to-decade.

Nonetheless, most authorities believe that diphtheria antitoxin does exhibit salutary effects on the course, complications and mortality of the disease, and that such effects are more pronounced the earlier in the course the antitoxin is given. However, it is clear that at best antitoxin fails to reduce mortality below about 5 percent.

Even less clear is the degree of effectivness of antitoxin in the prevention of diphtheria in exposed, susceptible individuals. The administration of an antimicrobial drug in therapeutic doses to exposed susceptible individuals avoids the use of horse serum and although not proven in controlled clinical trials, should be an effective alternative

regimen. Erythromycin appears to be the most effective; penicillin, lincomycin or rifampin are nearly as effective.

## Special Problems

Diphtheria antitoxin as used for the production of passive immunity in the treatment or prevention of diphtheria exhibits 2 special problems.

1. Diphtheria antitoxin is only partially effective in treatment, apparently because it neutralizes only circulating toxin. Toxin that has already left the circulation and is fixed to tissue is not inactivated, and no therapeutic agent has been identified that will interrupt the action of fixed toxin on tissue. Therefore, delayed therapy may not be effective.

2. Diphtheria antitoxin, comprising serum from horses immunized against the toxin, produces frequent symptomatic and occasional fatal hypersensitivity reactions.

## Recommendations

1. The limited therapeutic effectiveness of diphtheria antitoxin and doubts about its prophylactic efficacy plus the success of widespread active immunization of populations indicate the need to intensify the efforts toward active immunization of as many individuals as possible. Therefore, it is recommended that support for widespread public immunization programs be augmented. Such preventive programs are far more effective in reducing morbidity and mortality from diphtheria than is antitoxin, whether used therapeutically or prophylactically. A

widely immunized population would tend to eliminate the use of antitoxin and its attendant risk. (See Generic Statement on Diphtheria Toxoid and Tetanus and Diphtheria Toxoids for Adult Use (Td).)

2. Because passive immunization is still required for treatment of diphtheria in unimmunized individuals and occasionally in those apparently adequately immunized, consideration should be given to the development of diphtheria immune globulin of human origin.

3. Further information should be obtained regarding the possibility of a significant reduction in the reactivity of animal serum.

Basis for Classification

In the absence of controlled studies, difficult to obtain with this now rare life-threatening disease, the Panel could not insist on such evidence of efficacy. There is a sufficient body of historical data suggesting that diphtheria antitoxin is of some effect, albeit marginal, in the treatment and prophylaxis of diphtheria to justify classification in Category I.

BIBLIOGRAPHY

See Bibliography for Diphtheria Toxoid.

## SPECIFIC PRODUCT REVIEWS

### DIPHTHERIA ANTITOXIN MANUFACTURED BY BUREAU OF LABORATORIES, MICHIGAN DEPARTMENT OF PUBLIC HEALTH

No data have been provided by the manufacturer for diphtheria antitoxin, for which they are presently licensed. In the absence of any information from the manufacturer, the Panel can make no determination regarding the relative benefits and risks of this product.

Recommendations. The Panel recommends that this product be placed in Category IIIC and that the appropriate license be revoked for administrative reasons because this product is not marketed and there are insufficient data on labeling, safety, and effectiveness.

DIPHTHERIA ANTITOXIN MANUFACTURED BY ISTITUTO SIEROTERAPICO VACCINOGENO TOSCANO SCLAVO

1. Description. This diphtheria antitoxin is prepared from the plasma of horses hyperimmunized against diphtheria toxin. The plasma is semirefined by a process of enzymatic action, ammonium sulfate precipitation, heat and dialysis. The final product is sterilized by Millipore filtration and metacresol is added as a preservative to a concentration of 0.3 percent. The final product is marketed in 10,000 and 20,000 unit vials; the concentration is not specified.

2. Labeling--a. Recommended use/indications. This product is recommended for the treatment of diphtheria, and for the prevention of diphtheria in contacts who have not been previously immunized. For prevention, 10,000 units injected intramuscularly is recommended. For treatment, between 20,000 and 120,000 units, administered as a single dose, is recommended, with the larger doses being given to patients with more severe disease or disease of longer duration. It is recommended that approximately half of the dose be given intravenously and the rest intramuscularly.

Appropriate warnings are given about horse serum sensitivity and recommendations for intracutaneous or conjunctival testing for sensitivity are made. A satisfactory schedule is provided for the administration of antitoxin to individuals who display a positive sensitivity test. It is also stated that such individuals should not receive intravenous antitoxin.

b. Contraindications. The only contraindication listed is an intravenous injection to individuals with a positive sensitivity test.

3. Analysis--a. Efficacy--(1) Animal. This product meets Federal requirements.

(2) Human. No specific data are cited. Only general comments about confirmation of the efficacy of the product by results obtained in Italy and elsewhere since 1956 are stated in the manufacturer's submission to the Panel (Ref. 1).

b. Safety--(1) Animal. This product meets Federal requirements.

(2) Human. It is stated that many thousand vials have been distributed in the past 5 years without significant complaints regarding reactions.

c. Benefit/risk ratio. The methods of manufacture and the distribution of this antitoxin over the years indicate that it is comparable to other diphtheria antitoxins. The benefit-to-risk assessment of this product appears to be satisfactory for reasons cited in the Generic Statement.

4. Critique. This is an equine diphtheria antitoxin made according to accepted standards. It would appear to be as safe and as effective as any diphtheria antitoxin.

5. Recommendations. The Panel recommends that this product be placed in Category I and that the license(s) be continued with the stipulation that labeling be revised in accord with the recommendations of this Report.

DIPHTHERIA ANTITOXIN MANUFACTURED BY LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO.

No data have been provided by the manufacturer for diphtheria antitoxin, for which they are presently licensed. In the absence of any information from the manufacturer, the Panel can make no determination regarding the relative benefits and risks of this product.

Recommendations. The Panel recommends that this product be placed in Category IIIC and that the appropriate license be revoked for administrative reasons because this product is not marketed and there are insufficient data on labeling, safety, and effectiveness.

DIPHTHERIA ANTITOXIN MANUFACTURED BY MASSACHUSETTS PUBLIC HEALTH BIOLOGIC LABORATORIES

No data have been provided by the manufacturer for diphtheria antitoxin, for which they are presently licensed. In the absence of any information from the manufacturer, the Panel can make no determination regarding the relative benefits and risks of this product.

Recommendations. The Panel recommends that this product be placed in Category IIIC and that the appropriate license be revoked pending submission of evidence regarding the safety an effectiveness of this product.

DIPHTHERIA ANTITOXIN MANUFACTURED BY MERRELL-NATIONAL LABORATORIES, DIVISION OF RICHARDSON-MERRELL INC.

1. Description. Diphtheria antitoxin, U.S.P., as produced by Merrell-National Laboratories is prepared from the plasma of horses hyperimmunized with both diphtheria toxoid and toxin. The antitoxin content of the plasma is concentrated by ammonium sulfate precipitation and refined by partial pepsin digestion. The final diluent is physiologic saline and the preservative is 0.4 percent tricresol. The antitoxin is packaged in 20,000 unit vials with a concentration of at least 500 units per ml.

2. Labeling--a. Recommended use/indications. This product is recommended for the treatment of diphtheria and for prevention of diphtheria in exposed, susceptible individuals. The recommendations for its therapeutic use are complete, including precautions, appropriate regimens for sensitivity testing and desensitization, dosage schedules and the necessity for antimicrobial therapy.

Recommendations for prophylactic use in all exposed, susceptible individuals include sensitivity precautions, dosage, and emphasize subsequent active immunization. Serum sickness is described as a side effect. The package label is quite satisfactory.

b. Contraindications. None is specified, and it is stated that in individuals with diphtheria, antitoxin is mandatory.

3. Analysis--a. Efficacy--(1) Animal. Potency tests in animals are conducted according to Federal regulations.

(2) Human. No specific data are cited. The manufacturer states that early files on this product are no longer available. Excerpts from standard literature relating to diphtheria antitoxin are provided in the submission to the Panel (Ref. 2).

b. Safety--(1) Animal. This product is tested for total cresol, and for solids, pyrogenic activity and sterility according to Federal regulations.

(2) Human. No information is provided other than the absence of any reported medical complaints during the past 5 years, during which time thousands of doses were distributed.

c. Benefit/risk ratio. The benefit-to-risk assessment of this product appears to be satisfactory for reasons cited in the Generic Statement.

4. Critique. This product is still needed because of incomplete immunization of the United States populations and the continuing presence of diphtheria, and because a preparation produced in humans is not available. The package insert should reflect the preferability of erythromycin, lincomycin or penicillin over antitoxin for prevention of diphtheria in exposed, susceptible individuals.

5. Recommendations. The Panel recommends that this product be placed in Category I and that the license(s) be continued with the stipulation that labeling be revised in accord with the recommendations of this Report.

REFERENCES

(1) BER VOLUME 2112.

(2) BER VOLUME 2075.

GENERIC STATEMENT

Gas Gangrene Antitoxin

Gas gangrene is a serious and often fatal anaerobic infection of soft tissue, muscle, and sometimes blood. It is best known as a dreaded complication of injuries to soldiers in war-time, but occasionally occurs among civilians in peace time following trauma, or occasionally following surgery.

The etiologic agents of gas gangrene are the so-called "histotoxic" clostridia, including Clostridium perfringens, Clostridium novyi, Clostridium septicum, Clostridium histolyticum, Clostridium bifermentans, and Clostridium fallax. Clostridium perfringens is the most commonly recovered, and the best studied. All of these organisms require nearly complete anaerobiosis and a reduced oxidation-reduction potential for growth. In common with other clostridia such as Clostridium tetani, the histotoxic clostridia are widely distributed in nature, being readily found in the gastrointestinal tract of man and animals, as well as in soils.

It is generally believed that the various toxins produced by the histotoxic clostridia account for their rapid spread in tissue, and for the profound toxemia that is such a prominent part of the clinical picture of gas gangrene. Each species produces a number of extracellular toxins, including lecithinases, collagenases, proteinases, and deoxyribonucleases. The most widely studied of these toxins has been the alpha toxin of Clostridium perfringens, a lecithinase that injures cell membranes and alters capillary permeability. Although the acti-

vities of a few of these toxins have been carefully defined, the cause of the profound toxemia and extreme morbidity that accompanies clinical gas gangrene remains unclear. In addition to the toxins themselves, the toxemia has been attributed to release of the products of tissue necrosis, interference with enzyme systems, and the profound acidosis.

Active immunization using toxoids prepared from the histotoxic clostridia has not proven practicable on a large scale. When such toxoids are used to hyperimmunize horses, however, antitoxic activity does develop. Equine antitoxin has therefore been used in passive immunization in humans, both in the prophylaxis and treatment of gas gangrene.

## Nature of the Product

Polyvalent gas gangrene antitoxin is a preparation of hyperimmune serum from horses immunized against gas gangrene toxins.

## Production

Gas gangrene polyvalent antitoxins are produced from plasma of hyperimmunized horses. The crude plasma/saline mixture, at a pH of 3.9, is treated with pepsin and ammonium sulfate. "Digestion" is continued for 24 to 48 hours, at which time 75 to 80 percent of the protein will not coagulate on boiling. The material is filtered, the protein in the filtrate is precipitated by ammonium sulfate, and the precipitate is washed and suspended in phenolyzed distilled water with toluene and chloroform as additional preservatives. The resultant material contains mainly gamma and beta globulins.

The final product is diluted with sodium chloride solution and preserved with 1:20,000 phenylmercuric borate plus approximately 0.4 percent phenol. Each vial of the final product contains 10,000 units Clostridium perfringens antitoxin, 10,000 Clostridium septicum antitoxin, 3,000 Clostridium histolyticum antitoxin, 15,000 units Clostridium novyi antitoxin, and 15,000 units Clostridium bifermentans antitoxin.

Use and Contraindications

The main purpose of the administration of polyvalent gas gangrene antitoxin is to prevent death from toxemia in established cases of clostridial infection, and is therefore an adjunct to adequate surgery.

The recommended dosage schedule is approximately 50,000 units (2 vials) every 4 to 6 hours before or after surgery for a period of 24 to 48 hours. Administration is normally via the intravenous route, but it may be used intramuscularly as well.

It must be emphasized that prompt and adequate surgical debridement is the sine qua non in therapy of gas gangrene. Important adjunctive measures include careful management of fluid and electrolyte balance, and prompt antibiotic therapy, including large doses of penicillin G. Serotherapy with polyvalent gas gangrene antitoxin and hyperbaric oxygenation have been considered adjunctive measures whose relative merits are not clear.

Gas gangrene antitoxin is contraindicated in individuals with a history of sensitivity to horses, horse dander or horse serum, and should be given with extreme caution to anyone who has previously

received any injections containing horse serum.

## Safety

Federal regulations specify that polyvalent gas gangrene antitoxin must be tested to insure sterility, and contain an appropriate preservative in specified amount. The product must meet prescribed tests for freedom from pyrogenicity.

The most significant problem regarding the safety of polyvalent gas gangrene antitoxin relates to sensitivity to horse serum. Two types of hypersensitivity reactions occur; anaphylaxis and serum sickness. These reactions cannot always be predicted in advance by sensitivity testing, and may not be prevented by desensitization. Anaphylactic reactions to horse serum, fortunately the less common of the two, can occur without any known prior sensitization within a few minutes following injection, and most characteristically include cardiovascular collapse and shock. Even with prompt administration of epinephrine, death may occur in 10 percent or more of cases.

Serum sickness following horse serum occurs 6 to 21 days after an individual's first injection. The larger the dose of serum, the more likely is serum sickness to occur. The major manifestations of serum sickness are fever, arthritis, lymphadenopathy and urticaria. Symptoms persist for days or weeks. Fatalities are rare, except in instances of laryngeal edema. Rates of serum sickness following horse serum vary and are directly dose dependent. The frequency is approximately 1 percent per 1 ml of serum.

## Efficacy

The efficacy of polyvalent gas gangrene antitoxin has been extraordinarily difficult to assess with precision, owing to the fact that it is at best an adjunct in the management of gas gangrene.

For the prophylactic treatment of gas gangrene following traumatic injuries there is general agreement that polyvalent gas gangrene antitoxin is of no value. The work of MacLennan and MacFarlane, who studied the occurrence of gas gangrene among British troops during World War II, suggested that the incubation period of the disease might be lengthened by the administration of gas gangrene antitoxin, but clear evidence of efficacy in prophylaxis of gas gangrene cannot be found.

The mainstay of therapy of gas gangrene has been and continues to be prompt surgery, that includes complete removal of all infected tissue. Therapeutic regimens that have stopped short of such radical surgery have invariably failed, regardless of other adjunctive measures utilized. The adjunctive measures most often utilized include careful management of fluid and electrolyte balance, prompt antibiotic therapy, including large doses of penicillin G, passive immunization with polyvalent gas gangrene antitoxin, and hyperbaric oxygenation.

The best available data in support of therapeutic efficacy of polyvalent gas gangrene antitoxin derived from the British experiences in World War II, as summarized by MacLennan and MacFarlane. These studies were obviously not designed as rigidly controlled field trials, but did provide evidence that the combined use of surgery and antitoxin was approximately 40 percent more effective than surgery alone.

Data on the efficacy of antitoxin in the treatment of gas gangrene since world War II is scanty at best, wholly uncontrolled, and consists mostly of individual case reports or small series of cases.

Although it is difficult to dismiss entirely the experiences recorded by MacLennan, who felt that passive immunization with gas gangrene antitoxin was of distinct benefit in the management of gas gangrene, its role in management remains uncertain. Some or all of its apparent effectiveness during World War II may now have been minimized or eroded completely by emphasis on early diagnosis, prompt surgery, and other adjunctive and supportive therapy including antibiotics.

Current surgical opinion reflects these uncertainties. The manual "Control of Infection in Surgical Patients" edited by Altemeier, Burke, Pruitt, and Sandusky, states simply "gas gangrene antitoxin has been found to be of little or no value in the prevention of clinical gas gangrene."

## Special Problems

The major special problem identified is the lack of acceptable evidence of efficacy of polyvalent antitoxin in the management of clinical gas gangrene. The Panel sees no likelihood that such evidence will be forthcoming in the foreseeable future.

A second major problem in the evaluation of this product is the apparent lack of standardization of antitoxin unitage. International units of antitoxin are defined so that no two represent the same protective power, i.e., Clostridium novyi is approximately 100 times

greater than Clostridium perfringens, and Clostridium bifermentans is approximately 50 times greater than Clostridium perfringens. The protective power of "one vial" of the Lederle Laboratories Division's polyvalent gas gangrene antitoxin (pentavalent) in terms of mouse minimum lethal dose of toxin would be as follows:

| | |
|---|---|
| Clostridium perfringens | 500,000 to 700,000 |
| Clostridium septicum | 400,000 to 640,000 |
| Clostridium histolyticum | approx. to 135,000 |
| Clostridium novyi | approx. to 7,500,000 |
| Clostridium bifermentans | 2,850,000 to 5,700,000 |

Another aspect of this problem relates to the quantity of each of the antitoxins packed in a vial. This problem is illustrated in Table 1.

## Recommendations

The Panel recommends that further research be encouraged on the nature of the toxins produced by the histotoxic clostridia, and the mechanism of action of their effects on mammalian tissue.

## Basis for Classification

In the judgment of the Panel, there is not adequate evidence of efficacy of polyvalent gas gangrene antitoxin when used as recommended in either the prophylaxis or therapy of gas gangrene. Therefore, for this reason the Panel recommends that this product be classified in Category IIIB.

TABLE 1

Antitoxin Content (International Units)

| Author/Manufacturer | C. perfringens | C. septicum | C. novyi | C. histolyticum | C bifermentans | Recommended Dose |
|---|---|---|---|---|---|---|
| MacLennan (Ref. 1), MacFarlane (Ref. 2)/ | | | | | | |
| Medical Research | | | | | | |
| Council | 7,500 | 3,750 | 2,500 | -- | -- | ≥16,500 units (1 vial) |
| Gledhill/Burroughs | | | | | | |
| Welcome | 9,000 | 4,500 | 3,000 | -- | -- | 3 vials |
| Lindsey (Ref. 3), United States | | | | | | |
| National Standard; | | | | | | |
| Lederle | 9,000 | 4,500 | 9,000 | -- | -- | 12 ml/vial Dose not stated |
| Present product/ | | | | | | |
| Lederle | 10,000 | 10,000 | 1,500 | 3,000 | 1,500 | 2 vials |

REFERENCES

(1) MacLennan, J. D., "Anaerobic Infections of War Wounds in the Middle East," The Lancet. 2:123-126, 1943.

(2) MacFarlane, M. G., "The Therapeutic Value of Gas-Gangrene Antitoxin," British Medical Journal, 2:636-640, 1943.

(3) Lindsey, D., H. M. Wise, A. T. Knecht and H. E. Noyes, "Influenza of Route of Administration on Effectiveness of Clostridial Antitoxin," American Medical Association Archives of Surgery, 78:328-330, 1959.

SPECIFIC PRODUCT REVIEWS

GAS GANGRENE POLYVALENT ANTITOXIN MANUFACTURED BY LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY

1. Description. Gas gangrene polyvalent antitoxins are produced from plasma of hyperimmunized horses. After the antitoxic plasma is "refined and concentrated," it is diluted with sodium chloride solution and preserved with 1:20,000 phenylmercuric borate plus approximately 0.4 percent phenol. Each vial contains: 10,000 units Clostridium perfringens, 10,000 units of Clostridium septicum, 3,000 units of Clostridium histolyticum, 1,500 units of Clostridium novyi and 1,500 units of Clostridium bifermentans antitoxin.

The refining process involves pepsin/ammonium sulfate treatment of a crude plasma/saline mixture (pH 3.9). "Digestion" is continued for 24 to 48 hours, at which time 75 to 80 percent of the protein will not coagulate on boiling. The material is filtered, the protein in the filtrate is precipitated by ammonium sulfate, the precipitate is washed and suspended in phenolyzed distilled water with toluene and chloroform as additional preservatives. The resultant material contains mainly gamma and beta globulins.

2. Labeling--a. Recommended use/indications.

> ". . . to prevent death from toxemia in an established or suspected case of clostridial infection until adequate surgery and antibiotic therapy can bring the infection under control. The useful-

ness of this antitoxin to prevent clostridial infection is controversial but is generally considered to be of little or no value when given prophylactically."

The recommended dosage schedule is approximately 50,000 units (2 vials) every 4 to 6 hours before or after surgery for a period of 24 to 48 hours. Administration is normally intravenous but it may be used intramuscularly.

b. Contraindications. Sensitivity to horse serum, history of asthma, angioneurotic edema or other allergy.

3. Analysis--a. Efficacy--(1) Animal. This product meets Federal requirements.

Lindsey (Ref. 1) has demonstrated efficacy in extensively wounded goats when massive doses of trivalent antitoxin were employed, approximately 1,800 to 2,600 units of Clostridium perfringens antitoxin per kg.

(2) Human. The best available data derived from the British experiences in World War II.

(i) MacLennan (Ref. 2) demonstrated the following:

| | No antitoxin | | Antitoxin[1] | | |
|---|---|---|---|---|---|
| Drug Therapy | Cases | Death | Cases | Death | Differences |
| Sulfonamides | 28 | 22 (79%) | 58 | 19 (33%) | 46% |

[1]The average dose for survivors treated with antitoxin was 40,000 to 50,000 units. The composition of the antitoxin is not defined, but it is assumed to be that recommended by the Medical Research Council with 1 therapeutic dose containing 7,500 international units Clostridium perfringens antitoxin, 3,750 international units of Clostridium septicum and 2,500 international units of Clostridium novyi.

(ii) MacFarlane (Ref. 3) analyzed reports to subcommittee on anaerobic wound infections. The reports came from multiple sources between 1940 and 1943. Of 165 cases (not including those of MacLennan) 139 were classified as "toxic cases"; some received antitoxin, others had not. Results were as follows:

| No antitoxin | | Antitoxin | | |
|---|---|---|---|---|
| Cases | Death | Cases | Death | Differences |
| 25 | 21 (84%) | 114 | 58 (51%) | 33% |

From these studies they concluded that the combined use of surgery and antitoxin was more effective than surgery alone.

(iii) The MacLennan and MacFarlane studies which suggested effectiveness of gas gangrene antitoxin used preparations which differed in composition and which were administered in differing dosages. The Lederle gas gangrene antitoxin differs in composition from those used by both MacLennan and MacFarlane.

b. Safety--(1) Animal. This product meets Federal requirements.

(2) Human. Most reports contain no data on reactions; however, serum sickness would be anticipated. Frequency would be approximately 1 percent per 1 ml of serum.

c. Benefit/risk ratio. Benefit-to-risk considerations with reference to this product are not acceptable.

4. Critique. Major problems in the evaluation of this product have been discussed in the Generic Statement. The product is poorly standardized, and there is not adequate evidence of efficacy when used as recommended in either the prophylaxis or treatment of gas gangrene.

5. Recommendations. The Panel recommends that this product be classified as Category IIIB, and that the appropriate license be revoked owing to the lack of acceptable evidence of efficacy.

TETANUS AND GAS GANGRENE POLYVALENT ANTITOXIN MANUFACTURED BY LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO.

No data have been provided by the manufacturer for this product for which they were licensed at the time this review was undertaken. In the absence of any information from the manufacturer, the Panel can make no determination regarding the relative benefits and risks of this product.

Recommendations. The Panel recommends that this product be placed in Category IIIC and that the appropriate license be revoked pending submission of evidence regarding the safety and effectiveness of this product.

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(1) Lindsey, D., H. M. Wise, A. T. Knecht and H. E. Noyes, "Influence of Route of Administration on Effectiveness of Clostridial Antitoxin," American Medical Association Archives of Surgery, 78:328-330, 1959.

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(3) MacFarlane, M. G., "The Therapeutic Value of Gas-Gangrene Antitoxin," British Medical Journal, 2:636-640, 1943.

## GENERIC STATEMENT

### Pertussis Immune Globulin (Human)

The pathogenesis, symptomatology, complications and epidemiology of pertussis and its prevention with killed-bacterial vaccine have been described previously in this Report.

Serum therapy was initiated in the 1930's and early reports on the effect of convalescent human sera and hyperimmune animal sera in prophylaxis and therapy of pertussis were quite favorable. Subsequently a refined product, gamma globulin of human origin, was introduced and was similary accepted enthusiastically. Later controlled studies failed to demonstrate significant benefit.

Several factors may influence the effect of antibody therapy: 1. The site of the infection and access of antibody to the site; 2. whether antiserum alters the pathophysiologic effects of the organisms' reactive factors; and 3. the classes of immune globulin in convalescent serum which presumably contribute to recovery.

#### Description

Pertussis immune globulin is predominantly the immunoglobulin fraction from a pool of serum from human donors who have been hyperimmunized with pertussis vaccine. Earlier the product was sometimes obtained from persons who were hyperimmunized with vaccine following recovery from pertussis.

## Production

The source of this product is plasma from adults who have been repeatedly immunized with pertussis vaccine. This pertussis immune globulin is diluted with normal human immunoglobulin to achieve a standard concentration of protein. The donors are required to be free of causative agents of diseases that are not destroyed or removed by the processing methods, as specified by Federal regulations.

The plasma is fractionated by a cold alcohol method, yielding a preparation with over 90 percent of IgG. Thimerosal in dilution 1:10,000 may be added as a preservative. Pertussis immune globulin is submitted to standard tests for purity, sterility, safety and protein content according to Federal regulations. Up to this time there has been no standard of potency which has been correlated with human efficacy. The two products licensed in the United States at the present time are compared in an in vitro agglutination test to a reference serum.

## Use and Contraindications

The product has been recommended for intimately exposed children under 2 years of age who have not been vaccinated. The dose recommended by manufacturers is 1.25 to 1.5 ml intramuscularly, repeated in 5 to 7 days if exposure continues.

For treatment of infants with pertussis 1.25 ml intramuscularly for 3 to 5 doses, or 3 to 6.75 ml as a single dose is recommended. The product should not be administered intravenously.

Expert opinions as to the usefulness of pertussis immune globulin both in treatment and prophylaxis diverge. Thus the 1975 report of the American Public Health Association states that passive immunization is of no value in treatment or in prevention. However, the American Academy of Pediatrics which previously accepted its use in prophylaxis, in 1977 states that "There is no convincing evidence that Pertussis Immune Serum Globulin (Human) has any efficacy in preventing or treating pertussis, and its use is not recommended."

The product is contraindicated in individuals who are known to have an allergic response to immunoglobulin. Epinephrine should be at hand for treatment of rare reactions.

## Safety

This product must meet Federal regulations as to safety. Adverse reactions to immune globulins are rare, and consist of anaphylactic and allergic reactions. The greatest risk consists of inadvertent intravenous injection of aggregated immunoglobulin which leads to shock.

Manufacturers are required to record reported reactions.

## Efficacy

The use of pertussis immune globulin is empirical, because the nature of the protective factor in human serum is not known. However, the agglutinating antibody and/or a bactericidal antibody may play a role in protection. Furthermore, it is not clear whether protective factors are present in the IgG fraction. Some speculate that protection is located in the IgM fraction, because infants do not appear to obtain

passive immunity from their mothers. Since Bordetella pertussis infection is primarily an infection of the bronchial epithelium, it is also possible that the protective factor is located in the IgA fraction of the immunoglobulins. Pertussis immune globulin (human) can protect mice under experimental conditions, but its relation to human efficacy has not been determined.

Studies conducted in the 1930's and 1940's when pertussis was still a virulent disease with a relatively high mortality rate suggested a prophylactic and therapeutic effect from convalescent human sera and animal hyperimmune sera. Unfortunately, these studies were not adequately controlled and comparison groups outside the experimental setting were often utilized.

In the last decades a few controlled studies have been conducted with pertussis immune globulin. They did not demonstrate statistically significant differences between treatment and control groups. However, concurrent antimicrobial therapy may have masked any beneficial effect; it is also possible that the specific lots and dosage used were ineffective, and the numbers of study subjects were too few. At least in one study the dose was lower than the recommended one. Also, the stage of disease when the product was given has varied and the methods of allocation to study groups have not always been clearly described.

During the last decades, erythromycin and ampicillin have become the preferred methods for prophylaxis and treatment of pertussis.

Special Problems

1. Several studies, not adequately controlled, conducted in the 1930's and 1940's when pertussis was a more prevalent and virulent disease, provided evidence of therapeutic and prophylactic benefit from convalescent serum, human hyperimmune serum and rabbit hyperimmune serum. The initial experience with pertussis immune globulin (human) suggested similar effects, but more recent, well-controlled studies did not confirm this suggestion. Whether this indicates that alcohol fractionation of plasma in the preparation of immunoglobulin eliminates other protective components is unknown. It appears, however, that there is little evidence of efficacy of the current product.

2. No animal model or other laboratory technique for evaluation of potency has been directly related to efficacy in humans. The only animal model employed utilizes intracerebral injection of Bordetella pertussis bacteria into mice; a protective effect of pertussis immune globulin can be demonstrated. Other potentially useful models such as intranasal challenge of mice have been insufficiently studied.

3. Knowledge of the immune mechanisms to pertussis in humans, particularly as to class of immunoglobulin, and the role of humoral immunity, especially the role of bacteriocidal antibody, is rudimentary. The role of cell-mediated immunity is unknown.

4. Whereas the product appears relatively safe for the recipient, the practice of hyperimmunizing the donors with pertussis vaccine is not without risk.

## Recommendations

1. The available information is insufficient to classify pertussis immune globulin as effective. Further studies are required before such a decision can be made.

2. The Panel recommends that research be directed to identify the mechanism by which immunity to pertussis is acquired. Identification and characterization of protective antibodies, if such are present, are imperative to determine the value of pertussis immune globulin as presently constituted. Studies are also necessary to determine the value of other preparations derived from immune serum aimed at conferring passive immunity.

3. Animal models which closely resemble human infection should be sought, in order to study the pathogenesis and immune mechanisms of pertussis. A mouse model of respiratory infection already exists and deserves further exploration.

4. Clinical trials should be conducted with other immunoglobulin preparations that may have better experimental evidence for efficacy. Such studies could be carried out where the incidence of pertussis in childhood is high, or in special situations such as outbreaks among adults.

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SPECIFIC PRODUCT REVIEWS

PERTUSSIS IMMUNE GLOBULIN (HUMAN) MANUFACTURED BY CUTTER LABORATORIES, INC.

1. Description. This product is a solution of immunoglobulin prepared from venous blood of humans hyperimmunized with pertussis vaccine. It contains 16.5 percent ± 1.5 percent protein dissolved in 0.3 M glycine and preserved with 1:10,000 thimerosal. The pH is adjusted with sodium carbonate. Each 1-1/4 ml dose contains a quantity of immunoglobulin equivalent to approximately 25 ml of human hyperimmune plasma.

Fresh citrated plasma is collected by plasmapheresis and fractionated into components of plasma using the Cohn cold alcohol method. The pool of plasma is chosen on the basis of minimum pertussis titer and no regard is given to the number of donors. The final product solution is sterilized by filtration. Pertussis agglutination titers are determined but the standard used is not given. Donors, whose health status has been checked, receive a basic series of 3 injections of Eli Lilly and Company's pertussis vaccine, during a 12-month period and a fourth injection during a second 12-month period. A donor consent form is supplied.

2. Labeling--a. Recommended use/indications. The product is said to be indicated in the prophylaxis and treatment of pertussis. The dose is 1-1/4 ml given as soon after exposure as possible, and in therapy it is recommended that the same dose is repeated after 24 or 48 hours, sometimes again after 1 to 2 weeks. The product is given intramuscularly only, and not intravenously.

b. Contraindications. The product is contraindicated in individuals who are known to have an allergic response to immunoglobulin. There is a warning against intravenous use. Slight soreness may occur at the injection site; sensitization is extremely rare but may occur. There have been a few instances of angioneurotic edema, nephrotic syndrome and anaphylactic shock after injection.

3. Analysis--a. Efficacy--(1) Animal. Not applicable.

(2) Human. Several studies with pertussis immune globulin are cited in the submission to the Panel (Ref. 1), 7 of these utilized the product of this manufacturer. Whereas uncontrolled studies generally reported favorable results, the controlled studies failed to show any significant differences between control and treatment groups. The efficacy of the product, not only in treatment but also in prophylaxis, appears in doubt.

The only somewhat controlled study which reported favorable results is the one by Hatz (Ref. 2) who studied streptomycin with and without hyperimmune serum in treatment of pertussis. However, the conclusions appear not to be statistically validated.

It is disconcerting that controlled studies, generally carried out after 1950 when pertussis had become a relatively mild disease and effective antibiotics were available, all report a lack of statistically significant benefit from pertussis immune globulin. On the other hand, uncontrolled or poorly controlled studies carried out with whole immune serum in the 1930's and 1940's suggested great benefit, especially in

prophylaxis. If the protective antibody is found in the IgM fraction of the immune globulin as suggested in "Infectious Diseases" by Krugman and Ward (Ref. 3), how can the IgG (which is the principal content of hyper-immune globulin) be of any help? Maternally acquired immunoglobulin is known not to be protective.

b. Safety--(1) Animal. This product meets Federal requirements.

(2) Human. Several clinical trials report no adverse effects. Rare instances of angioneurotic edema, nephrotic syndrome and anaphylactic shock are listed as possible adverse reactions. No data from the complaint file are submitted.

c. Benefit/risk ratio. The benefits of this product both in prophylaxis and treatment are in doubt, although there is little risk (isoimmunization, allergic reactions).

4. Critique. This is a well documented submission except that data from the manufacturer's complaint file were not submitted. It is unclear how many donors make up the pool for pertussis immune globulin (the Bureau of Biologics requires a minimum of 10 individuals). The label states that the donors are given Cutter Laboratories' pertussis vaccine, other sections of the manufacturer's submission indicate that Eli Lilly's vaccine is used. Information on adverse reactions to repeated administration of pertussis vaccine in adults and the procedure utilized in the production of pertussis immune globulin (human) should be developed. This information should include data on the type of vaccine used. The agglutination test, including standards, is not

described. The submission contains a thorough listing of human studies of pertussis immune globulin, including several of the manufacturer's own product. Their own interpretation of these studies is that the product is efficacious. It is unfortunate that this conclusion is based on uncontrolled studies, and not on the controlled ones, which do not prove any statistically significant benefits.

5. Recommendations. The Panel recommends that this product be placed in Category IIIA and that the appropriate license be continued for a period not to exceed 3 years during which time the manufacturer shall develop data regarding the efficacy of this product.

PERTUSSIS IMMUNE GLOBULIN (HUMAN) MANUFACTURED BY TH[illegible]OL LABORATORIES, INC. HYLAND DIVISION

1. Description. This product is a 16.5 (± 1.5) percent solution of the immunoglobulin fraction (Cohn Fraction II) of the serum of healthy adults hyperimmunized with pertussis vaccine. The solution is made isotonic and stabilized with 0.3 molar glycine. It contains 0.1 percent sodium chloride and 0.01 percent thimerosal as a preservative. Cryoprecipitate is removed by centrifugation and reserved for other use. Fraction II is obtained from Fraction I, II, III by the Cohn method with some modifications. Donors are given 3 doses (0.5 ml) of pertussis vaccine subcutaneously at weekly intervals, the fourth dose is given after 4 weeks, and later doses are given at 4 week intervals as long as the donor remains on the program. Plasmapheresis is performed twice weekly.

The product is available in 1.5 ml single dose vials.

2. Labeling--a. Recommended use/indiciations. In prophylaxis, one 1.5 ml dose of pertussis immune globulin (human) is recommended for a child as soon after exposure as possible. A second dose, 1 week after the first, is desirable. If use of the globulin is delayed more than 1 week after exposure, larger doses should be given at 1 to 2 week intervals.

In treatment, for children already showing symptoms of pertussis, one 1.5 ml dose should be given as soon as possible, with additional doses at 2 day intervals until recovery has begun. For critically ill

children, the initial dose might well be doubled. In cases of pertussis pneumonitis, the globulin treatment may be supplemented with suitable sulfonamide or antibiotic therapy.

It is clearly stated that the product should be given intramuscularly and not intravenously.

b. Contraindications. None are listed. Under reactions the remote possibility of serum sickness and anaphylaxis are mentioned, as well as local tenderness and stiffness. A warning against intravenous injection is given.

3. Analysis--a. Efficacy--(1) Animal. Not applicable.

(2) Human. The manufacturer's submission to the Panel (Ref. 4) cites the literature of pertussis immune globulin, but they appear not to have conducted any field tests of their own product. The product is tested for potency by measurement of agglutination titers. The agglutination titers of the lot under test, a house reference lot, and the starting plasma pool are determined, using as the antigen a commercially available licensed pertussis vaccine, always from the same manufacturer. The lot under test must show at least 16 times concentration of antibody over the starting plasma pool (i.e., 4 doubling dilutions difference) and the house reference lot must show the same titer as it showed in previous tests, plus or minus 1 doubling dilution. No reference or standard from the Bureau of Biologics is being utilized.

b. Safety. This product is tested for purity, residual moisture, pyrogens, electrophoretic purity, "general safety," and stability.

(1) Animal. This product meets Federal requirements.

(2) Human. No data on human safety for this specific product were supplied other than from the general literature. No data from the manufacturer's complaint file were submitted.

c. Benefit/risk ratio. The benefits of this product both for use in prophylaxis and treatment are questionable. Several uncontrolled studies report beneficial results, but the controlled studies, even those investigating the prophylactic use (Morris (Ref. 5) and Place (Ref.6)) report no significant differences between patients given pertussis immune globulin and other material. The risks are minimal, but allergic reactions and isoimmunization have to be considered.

4. Critique. The most difficult problem is to determine if the current literature supports the belief that the use of pertussis immune globulin is effective in prophylaxis, let alone treatment of pertussis. The manufacturer's own product has not been field tested; however, such a test would be very difficult to institute. Data from complaint files are lacking. The Bureau of Biologics does provide a United States standard antipertussis serum, and the provisional requirements state that each lot of pertussis immune globulin shall contain a pertussis antibody level of not less than 500 pertussis units per vial compared with this standard. Information on adverse reactions to repeated administration of pertussis vaccine in adults, a procedure utilized in the production of pertussis immune globulin (human), should be developed. This information should include data on the type of vaccine used.

Since sulfonamides are not the first choice in treatment of pertussis, the advice regarding supplementary treatment should be reworded: substitute "sulfonamide or antibiotic therapy" with "antimicrobial therapy."

5. Recommendations. The Panel recommends that this product be placed in Category IIIA and that the appropriate license be continued for period not to exceed 3 years during which time the manufacturer shall develop data regarding the efficacy of this product.

REFERENCES

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(4) BER VOLUME 2106.

(5) Morris, D. and J. C. McDonald, "Failure of Hyperimmune Gamma Globulin to Prevent Whooping Cough," Archives of Diseases of Children, 32:236-239, 1957.

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GENERIC STATEMENT ON TETANUS ANTITOXINS

Tetanus is an acute disease of the nervous system caused by infection with the tetanus bacillus, Clostridium tetani, which produces an extremely potent neurotoxin that is lethal to man in miniscule amounts (approximately 7 millionths of a milligram). The tetanus bacillus also produces lesser reactive substances. The disease is of major importance, killing perhaps 1 million people worldwide annually. The tetanus bacillus is probably primarily a resident of the intestinal tract of various animals, but spores are widely distributed in soil and dirt, and when carried into devitalized injured human tissue that is low in oxygen, the spore form of the bacillus can germinate, liberate toxin and hence cause the disease. The disease can be prevented by immunization with tetanus toxoid. Immunization is indicated for everyone, since natural immunity, if it exists at all, is exceedingly rare in man; not even the disease itself produces immunity in those who recover from it.

In the 1890's, tetanus antitoxin was developed, primarily in horses, by hyperimmunization--first by injection of slowly increasing amounts of tetanus toxin, and later, when it became available, by sequential injections of tetanus toxoid. The serum from such animals contained varying amounts of antibody capable of neutralizing tetanus toxin in experimental animals; therefore it has been used on a worldwide basis ever since both for the prophylaxis of tetanus in unimmunized persons thought to be exposed to the disease, and for treatment of the disease.

Both the safety and efficacy of tetanus antitoxin of animal origin have been the subject of concern and disagreement ever since its introduction, because of the frequency of reactions--not infrequently severe and sometimes fatal--following the injection of horse serum in sensitive individuals, and because unequivocal data regarding its efficacy have never become available. Substitution of antiserum prepared in cattle or sheep did not solve either problem, and during the past 15 years attention has been turned to the preparation of concentrated antitetanus antibody solutions from immunized or hyperimmunized human donors. The human preparation, designated tetanus immune globulin, has eliminated the problem of reactions to heterologous serum, but the problem of efficacy remains unsettled. Nevertheless, the theoretical considerations and the clinical impression that either or both of these products are of value have led to their very general use, for prophylaxis of tetanus in previously unimmunized persons incurring a risk of contracting tetanus, and in the treatment of clinical tetanus.

## Nature of Product

Tetanus antitoxin consists of the partially purified globulin fraction from the serum of animals (generally horses) hyperimmunized with multiple sequential doses of tetanus toxoid, and sometimes toxin as well. Potency in units is determined by reference to the U.S. standard antitoxin. Antitoxin of bovine or ovine origin is similar except for minor differences in the predominant type of antitoxin-containing globulin.

Tetanus immune globulin is the gamma globulin fraction from a pool of human donors who have either been selected because they already possess a sufficiently high serum antitoxin level against tetanus toxin, or else have been hyperimmunized so that their serum antitoxin level is suitably high.

## Production

For the production of tetanus antitoxin, the best responders are selected from a number of horses that have been given several properly spaced injections of tetanus toxoid, and further immunized until test bleeding showed that their serum antitoxin level is high enough to yield a concentrated antitoxin of acceptably high titer, e.g., 1,500 units or more per ml. Present day harvesting of serum is done by plasmapheresis, collecting 8 to 9 liters of blood and retransfusing the separated cells, on a regular schedule such as every 2 weeks. The plasma is fractionated, usually by precipitation of the antitoxic antibodies with ammonium or sodium sulfate, yielding a mixture of proteins which contains a high proportion of the antitoxic globulin which is, in the horse, largely a beta-globulin. The precipitate is reconstituted, dialyzed and adjusted to yield approximately a 20 percent concentration of serum proteins. Further purification of the original serum is usually carried out under specified conditions of pepsin digestion, which hydrolyses much of the nonglobulin protein present, yielding a preparation with fewer nonspecific proteins and a higher ratio of beta-globulin, modified by digestion but still fully active against toxin. In practice, the proportion of specific antitoxin in the usual product is probably about 1 to 2 percent.